Indian Journal of Research in Homoeopathy

Volume 20 | Issue 1 Article 4

25-3-2026

Screening of antiurolithiatic activity of different formulations of homoeopathic medicines using calcium oxalate crystallisation assay

Loganathan G
Vinayaka Mission's Homoeopathy Medical College and Hospital, Tamil Nadu, India, loganathan23897@gmail.com
Satesh T
Vinayaka Mission's Homoeopathy Medical College and Hospital, Tamil Nadu, India, satheshmdmd@gmail.com
Author(s) ORCID Identifier:
Loganathan G: https://orcid.org/0009-0000-8349-690X

How to cite this article

G Loganathan, T Satesh. Screening of antiurolithiatic activity of different formulations of homoeopathic medicines using calcium oxalate crystallisation assay. *Indian J Res Homoeopathy.* 2026;20(1):30–44.

Screening of antiurolithiatic activity of different formulations of homoeopathic medicines using calcium oxalate crystallisation assay

Abstract

Background: Homoeopathic medicines such as *Aerva lanata, Hydrangea arborescens, Berberis vulgaris, and Sarsaparilla* are commonly used to treat kidney stones. Scientific research is needed to understand their effects on calcium oxalate crystallisation, a key factor in urolithiasis. Objective: The study evaluated the in vitro effects of *Aerva Lanata, Hydrangea Arborescens, Berberis Vulgaris, and Sarsaparilla* in Q, 6C, and 30C potencies on calcium oxalate crystallisation. It aimed to identify which potency had the strongest inhibitory effect compared to Ethanol (90%). Methods: Calcium oxalate (CaOx) crystals were synthesised by mixing calcium chloride and sodium oxalate, then cooled to 37°C to achieve a concentration of 0.8 mg/mL in Tris buffer (pH 6.5). Homoeopathic remedies (*Aerva lanata, Berberis vulgaris, Hydrangea arborescens*, and *Sarsaparilla* in Q, 6 C, and 30 C potencies) and ethanol (90%) were tested at concentrations of 1000 to 10 µg/ml. After 24 hours at 37°C, turbidity was measured, and crystal morphology was examined under an inverted light microscope. Results: *Aerva lanata Q, Aerva lanata 6C*, and *Berberis vulgaris Q* significantly inhibited stone formation compared to the ethanol control group, with Aerva Lanata Q demonstrating the most pronounced effect. Light microscopy revealed diverse crystal morphologies, underscoring the role of Calcium Oxalate crystals in kidney stone formation. Conclusion: *Aerva lanata* and *Berberis vulgaris* show potential in inhibiting calcium oxalate crystal formation, suggesting a role in preventing urolithiasis.

Acknowledgments and Source of Funding

Financial support : Self funding Acknowledgement: The authors are grateful to Trichy Research Institute of Biotechnology Pvt. Ltd., Trichy– 620018, Tamil Nadu for the technical support and facilities provided. We also thank Dr. K. Rajamanickam, Dr. A. Vettrivel and Dr. SP. Mary Adharshna for the support rendered.

Scan the QR to view the full-text article on the journal website

ORIGINAL ARTICLE

Screening of antiurolithiatic activity of different formulations of homoeopathic medicines using calcium oxalate crystallisation assay

Loganathan G, Satesh T

Vinayaka Mission's Homoeopathy Medical College and Hospital, Tamil Nadu, India

ABSTRACT

Background: Homoeopathic medicines such as *Aerva lanata*, *Hydrangea arborescens*, *Berberis vulgaris*, and *Sarsaparilla* are commonly used to treat kidney stones. Scientific research is needed to understand their effects on calcium oxalate crystallisation, a key factor in urolithiasis. **Objective:** The study evaluated the in vitro effects of *Aerva Lanata, Hydrangea Arborescens, Berberis Vulgaris*, and *Sarsaparilla* in Q, 6C, and 30C potencies on calcium oxalate crystallisation. It aimed to identify which potency had the strongest inhibitory effect compared to Ethanol (90%). **Methods:** Calcium oxalate (CaOx) crystals were synthesised by mixing calcium chloride and sodium oxalate, then cooled to 37 ˚C to achieve a concentration of 0.8 mg/mL in Tris buffer (pH 6.5). Homoeopathic remedies (*Aerva lanata, Berberis vulgaris, Hydrangea arborescens,* and *Sarsaparilla* in Q, 6 C, and 30 C potencies) and ethanol (90%) were tested at concentrations of 1000 to 10 μg/ml. After 24 hours at 37 ˚C, turbidity was measured, and crystal morphology was examined under an inverted light microscope. **Results:** *Aerva lanata Q, Aerva lanata 6C*, and *Berberis vulgaris Q* significantly inhibited stone formation compared to the ethanol control group, with *Aerva Lanata* Q demonstrating the most pronounced effect. Light microscopy revealed diverse crystal morphologies, underscoring the role of Calcium Oxalate crystals in kidney stone formation. **Conclusion:** *Aerva lanata* and *Berberis vulgaris* show potential in inhibiting calcium oxalate crystal formation, suggesting a role in preventing urolithiasis.

Keywords: Crystal aggregation, Homoeopathy, Renal calculus

Introduction

Nephrolithiasis, or urolithiasis, refers to the formation of stones in the urinary tract due to the supersaturation of minerals in the urine, leading to crystal formation and aggregation.[1] Between 5 and 12% of Indians have urolithiasis, with greater incidences noted in the northern regions that make up the "stone belt." High recurrence rates also greatly increase the cost of healthcare.[2] The most common urinary stones, making up about 75% of cases, are calcium-containing, including pure calcium oxalate (45%), pure calcium phosphate (5%), or a combination (5%), with mineral metabolism being a key contributing factor.[3] Urolithiasis is a complex condition resulting from various processes, including high-concentration mechanisms, the formation, growth, accumulation, and retention of calculi in renal tubules; nucleation occurs when urine becomes supersaturated, leading to the development of solid crystals.[4]

Aerva lanata (L.) has been traditionally used in India as a diuretic and for the treatment of urolithiasis, owing to its rich phytochemical content, which provides diuretic, antioxidant, and anti-inflammatory benefits. It lowers calcium, phosphate, and oxalate levels while increasing magnesium in the urine, thereby inhibiting stone formation by promoting calcium dissolution. Consequently, *Aerva lanata* is noted for its strong diuretic properties and its ability to hinder the nucleation stage of stone formation.[5] *Aerva lanata*

How to cite this article: G Loganathan, T Satesh. Screening of antiurolithiatic activity of different formulations of homoeopathic medicines using calcium oxalate crystallisation assay. *Indian J Res Homoeopathy.* 2026;20(1):30–44.

Received 10 November 2024; Accepted 20 February 2026.

E-mail address: loganathan23897@gmail.com (Loganathan G).

https://doi.org/10.53945/2320-7094.2301

reduces the excretion of calcium and oxalate, potentially by suppressing oxalate production, making it an effective antilithic agent. This reduction lowers urine supersaturation with calcium oxalate, thereby decreasing the likelihood of stone formation.[6]

Berberis vulgaris L, containing the alkaloid berberine, has antioxidant, anti-inflammatory, and diuretic properties that help prevent oxidative stress and inhibit renal calculus formation. It promotes the excretion of calcium oxalate crystals, reducing the risk of kidney stones.[5] *Berberis vulgaris,* widely used in Homoeopathy for kidney discomfort and stone removal, contains root-bark components that may prevent renal stone formation through antioxidant activity and the inhibition of calcium oxalate crystallisation. This supports its potential as a source of new antiurolithic drugs and justifies its therapeutic use in nephrolithiasis.[7]

Hydrangea arborescens, also known as "the stone breaker" or "seven-barks," is a well-known homoeopathic remedy for treating urinary tract calculi, helping to break down stones and alleviate associated symptoms.[8] Boericke describes *Hydrangea* as a remedy for urinary issues, including white salt deposits, renal colic, and calculus, and addresses symptoms such as back pain, urethral burning, and difficult urination. It also helps with enlarged prostate and gravel deposits.[9] Studies show that *Hydrangea* can dissolve calcium oxalate stones and prevent crystalluria, though the exact active components remain unclear. Homoeopathic medicines have been proven effective in delaying and inhibiting stone formation, supporting their use in urolithiasis.[10]

Sarsaparilla's effects are linked to its steroids and saponins, which enhance the absorption of other herbs. Historically, it has been used to boost bioavailability and potency in herbal formulas.[11] Boericke describes "*Sarsaparilla*" in homoeopathic literature as a remedy for scanty, slimy, flaky, sandy, and bloody urine, along with symptoms like kidney colic and extreme discomfort after urination. It addresses issues such as bladder soreness and enlargement, painful urination in infants, and a weak urine stream with meatus pain.[9] *Sarsaparilla*, noted in Homoeopathic Materia Medica and Repertory, acts as a diuretic for kidney stones and has been shown effective in dilutions as a preventive measure. It serves as a crystallisation inhibitor for calcium phosphate and calcium oxalate, proving to be more beneficial for calcium oxalate stone disease than for calcium phosphate.[12]

Most patients experience kidney stone recurrence, although repeat surgery might not be the best course of action. Research indicates that using homoeopathic medications to treat calculi may have a good effect on their dissolution or expulsion.[13]

Despite the traditional use of *Aerva lanata, Berberis vulgaris, Hydrangea arborescens,* and *Sarsaparilla* in Homoeopathy for renal calculi, comparative experimental evidence across different potencies is scarce. Since calcium oxalate crystallisation is the primary mechanism in most kidney stones,[1] this study evaluates and compares their inhibitory effects in Q, 6C, and 30C potencies using an in vitro model to provide scientific support for their therapeutic application.

Material and methods

Homoeopathic medicines *Aerva lanata* (Q, 6C, 30C), *Berberis vulgaris* (Q, 6C, 30C), *Hydrangea arborescens* (Q, 6C, 30C), *Sarsaparilla* (Q, 6C, 30C) and Ethanol 90% were procured from a standard pharmaceutical company through RG Herbal Medikalaya, Coimbatore, Tamil Nadu.

Other parameters of the study and assay were done in Tri-Biotechnology Private Limited, Trichy, Tamil Nadu.

Calcium oxalate method

The analysis of calcium oxalate (CaOx) crystals in experimental kidney stones was conducted using the methodology formed by Mazni Abu Zarin *et al.*, with a small adjustment. CaOx monohydrate crystals were synthesised through the combination of $CaCl_2$ (50 mmol/L) and Na_2CO_2 (50 mmol/L) at equivalent concentrations. The solutions were equilibrated in a water bath at 60˚C for 1 hour to facilitate the development of CaOx monohydrate crystals. Before evaporation, the crystals were chilled to 37°C. The CaOx monohydrate crystals were synthesised upon reaching a final concentration of 0.8 mg/ml in a Tris buffer solution (Tris 0.05 mol/L and sodium chloride 0.15 mol/L) under physiological conditions at a pH of 6.5.[14]

Samples of *Aerva lanata* (Q, 6C, 30C), *Berberis vulgaris* (Q, 6C, 30C), *Hydrangea abrorescens* (Q, 6C, 30C), *Sarsaparilla* (Q, 6C, 30C) and Ethanol 90% (dose calculated based on dilution from stock 100% ethanol using standard v/v conversion: 90 ml ethanol + 10 ml distilled water) were added to a solution of CaOx monohydrate crystals at different concentrations (10, 50, 100, 250, 500, and 1000 μg/ml). The final volume used per reaction was 200 μg/mL in each well, ensuring equal exposure to concentration across treatments. The solution was incubated at 37 °C for 24 hours. Using a microplate reader (Thermo

Table 1A. Percentage Inhibition (%) of CaOx Aggregation – *Aerva lanata*.

Concentration (μg/mL)	*Q (Mean ± SD)*	*6C (Mean ± SD)*	*30C (Mean ± SD)*
10	64.98 ± 0.92	59.27 ± 0.52	26.50 ± 4.13
50	71.49 ± 2.01	66.77 ± 1.03	37.70 ± 0.46
100	78.25 ± 2.49	70.45 ± 1.19	42.34 ± 2.02
250	82.03 ± 0.43	72.08 ± 0.14	45.80 ± 0.55
500	83.78 ± 0.43	73.24 ± 1.01	48.81 ± 0.49
1000	*86.48 ± 1.25*	*77.26 ± 1.31*	*57.44 ± 6.97*

Table 1B. Percentage Inhibition (%) – *Berberis vulgaris*.

Concentration (μg/mL)	*Q*	*6C*	*30C*
10	0.00 ± 0.00	17.35 ± 2.90	2.04 ± 1.16
50	15.10 ± 2.54	25.18 ± 2.98	11.53 ± 1.08
100	23.76 ± 1.04	29.53 ± 1.94	13.27 ± 0.33
250	34.27 ± 4.85	33.33 ± 0.00	14.27 ± 0.65
500	56.09 ± 2.01	36.28 ± 0.28	21.43 ± 4.10
1000	***73.95 ± 1.25***	***65.00 ± 3.39***	***42.75 ± 13.03***

Table 1C. Percentage Inhibition (%) – *Hydrangea arborescens*.

Concentration (μg/mL)	*Q*	*6C*	*30C*
10	15.49 ± 2.17	16.13 ± 1.19	6.57 ± 1.68
50	33.30 ± 1.40	23.73 ± 1.74	15.02 ± 2.64
100	35.69 ± 0.65	27.73 ± 1.21	21.53 ± 1.73
250	41.16 ± 1.55	30.44 ± 1.26	23.95 ± 1.00
500	49.22 ± 3.86	38.86 ± 0.49	29.82 ± 3.30
1000	***55.15 ± 2.05***	***41.94 ± 1.78***	***38.95 ± 1.76***

Fisher Scientific, USA; Model: Multiskan SkyHigh, Cat. No. 51119700) to measure turbidity, the aggregation activity was estimated in the presence of the extract at 620 nm and compared with the control.

The experimental procedure was conducted in triple. The percentage of aggregation inhibition by the plant extract was calculated as follows:

$$\%\text{inhibition} = \text{OD control} - \text{OD test}/\text{OD control} \times 100$$ [15]

Light microscopic studies

Crystals of CaOx were created for light microscopic analysis using the procedure described by Mazni Abu Zarin *et al.*[14] A solution of CaOx monohydrate crystals was supplemented with homoeopathic medicinal samples of *Aerva lanata* (Q, 6C, 30C), *Berberis vulgaris* (Q, 6C, 30C), *Hydrangea arborescens* (Q, 6C, 30C), and *Sarsaparilla* (Q, 6C, 30C) were tested. The mother tincture (Q) was used at stock strength and diluted in distilled water to obtain final concentrations of 10, 50, 100, 250, 500, and 1000 μg/mL. For 6C and 30C potencies, drops of each potency (corresponding to 0.05 ml) were added to the reaction mixture and adjusted to the same final volumes, ensuring equal exposure across treatments. Ethanol 90% was included as a control at equivalent concentrations. The solution was incubated for 24 hours at 37 °C. Every individual droplet of the suspension was uniformly spread across a glass slide, positioned on top of a cover slip, and analysed using a light microscope, more precisely, a light inverted microscope. The slide was thereafter captured in photomicrographs at magnifications of ×10 and ×40.[10]

Statistical analysis

All experiments were performed in triplicate, and the results are expressed as mean ± standard deviation (SD). The percentage inhibition values of each treatment were compared using one-way analysis of variance (ANOVA) to determine overall significance, followed by Tukey's Honest Significant Difference (HSD) post-hoc test for pairwise comparisons. The statistical significance threshold was set at $p < 0.05$. IC_{50} values were calculated using nonlinear regression analysis. Statistical analyses were conducted using GraphPad Prism version X.X software.

Data were assessed for normality using the Shapiro–Wilk test and for homogeneity of variances using Levene's test. All treatment groups followed a normal distribution ($p > 0.05$) except the ethanol control group ($p = 0.0288$), and variances were homogeneous ($p = 0.347$). Therefore, one-way ANOVA

Table 1D. Ethanol control.

Concentration (μg/mL)	*% Inhibition (Mean ± SD)*
10	0.00 ± 0.00
50	5.47 ± 1.53
100	10.86 ± 1.12
250	17.48 ± 4.24
500	34.51 ± 5.53
1000	***37.03 ± 4.07***

followed by Tukey's HSD post-hoc test was applied, as ANOVA is robust to minor deviations from normality.

Results

At 1000 μg/ml, *Aerva lanata* Q, *Aerva lanata* 6C, and *Berberis vulgaris* Q showed significantly higher inhibition compared to the control ($p < 0.01$). The 1000 μg/ml concentration for Q potency was obtained by preparing a stock solution of the mother tincture (Q) and serially diluting with Tris buffer to achieve the desired mass concentration. For 6C and 30C potencies, the calculation was based on the standard centesimal dilution principle (1 part in 100 repeated 6 or 30 times, respectively). For

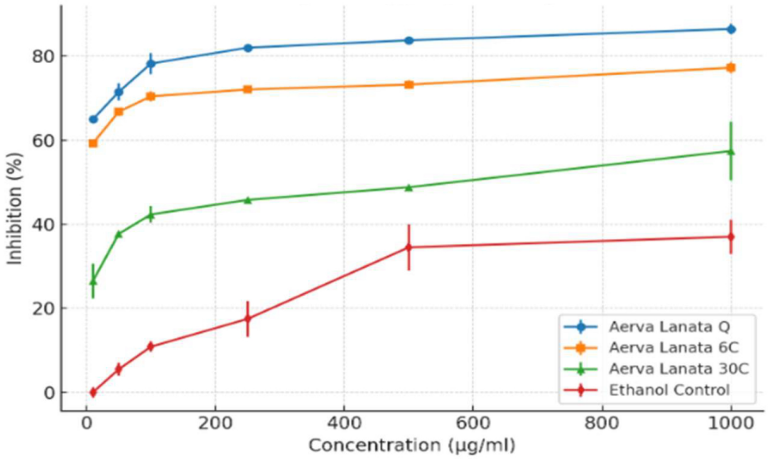


Fig. 1. Inhibition of calcium oxalate (CaOx) crystal aggregation by *Aerva lanata* (Q, 6C, 30C) compared with ethanol control. Values represent **mean ± SD** of three independent experiments performed in triplicate. Error bars indicate SD. Statistical differences from control were assessed by one-way ANOVA followed by Tukey's HSD test ($p < 0.05$, $p < 0.01$, $p < 0.001$).

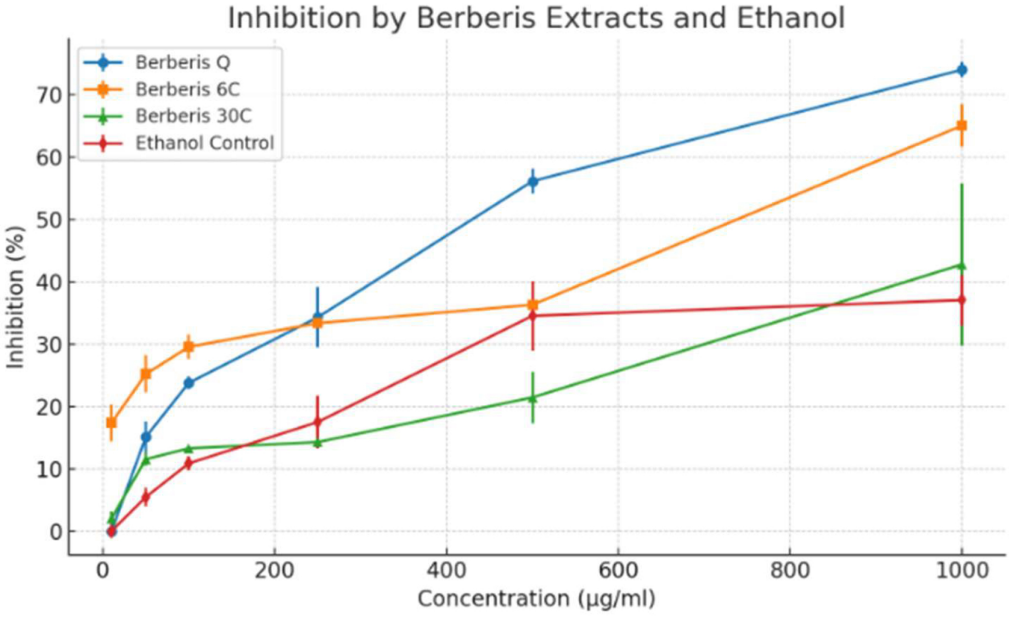


Fig. 2. Inhibition of CaOx crystal aggregation by *Berberis vulgaris* (Q, 6C, 30C) compared with ethanol control. Values represent mean ± SD of three independent experiments performed in triplicate. Error bars indicate SD. Significance relative to control determined by one-way ANOVA with Tukey's HSD test.

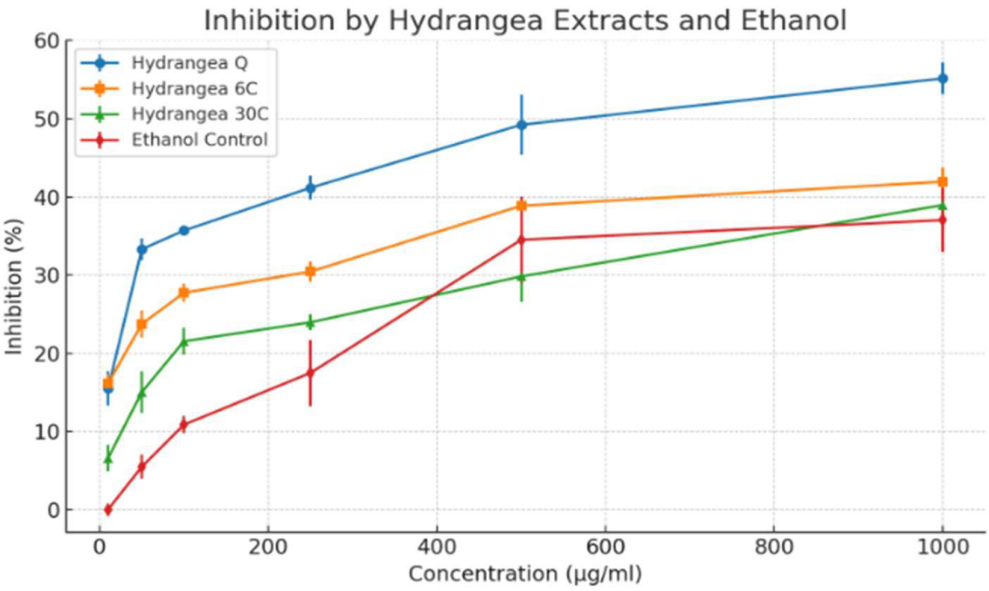


Fig. 3. Inhibition of CaOx crystal aggregation by *Hydrangea arborescens* (Q, 6C, 30C) compared with ethanol control. Mean ± SD from triplicate experiments. Error bars represent SD. Statistical analysis by one-way ANOVA and Tukey's HSD test.

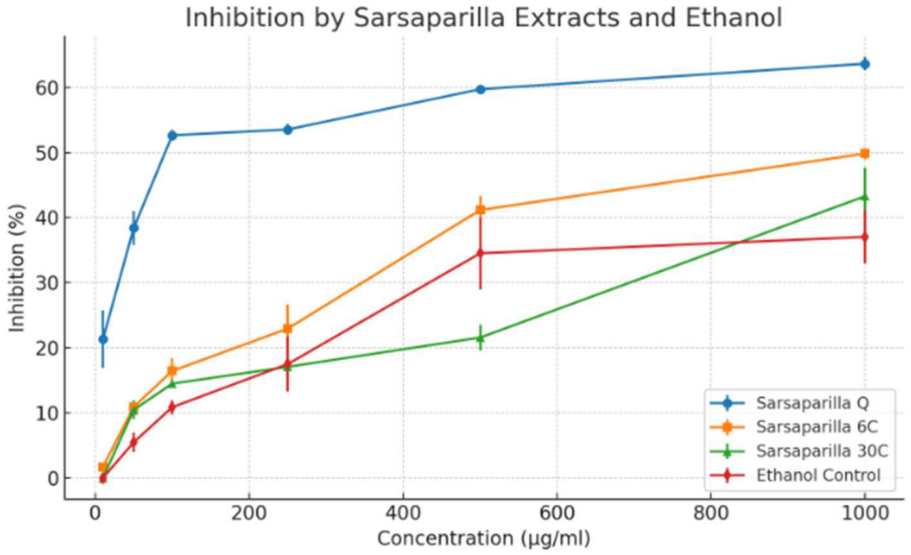


Fig. 4. Inhibition of CaOx crystal aggregation by *Sarsaparilla* (Q, 6C, 30C) compared with ethanol control. Values represent mean ± SD from three experiments. Error bars show SD. Differences from control were tested by one-way ANOVA followed by Tukey's HSD test.

the assay, 0.05 mL of each potency was taken and further adjusted with Tris buffer to achieve a final concentration of 1000 μg/mL in the test solution, ensuring comparability with the Q potency and ethanol control, with *Aerva lanata* Q being the most potent (86.48%). The percentage inhibition values of these medicines are presented in Tables 1A to 1D and Figs. 1 to 4 at different concentrations (10, 50, 100, 250, 500, 1000 μg/ml). The analysis was conducted using ANOVA, followed by Tukey's (HSD) post-hoc analysis to compare the mean inhibition percentages across the different groups. Significant inhibition levels were observed for the following: *Aerva lanata* Q (86.48%), *Aerva lanata* 6C (77.26%), *Berberis vulgaris* Q (73.95%), *Berberis vulgaris* 6C (65.00%), *Sarsaparilla Q* (63.64%), *Aerva lanata* 30C (57.44%), and *Hydrangea arborescens* Q (55.15%). The inhibition effect was specifically assessed at a concentration of 1000 μg/ml, with statistical analysis revealing significant differences between the homoeopathic treatments and the control (ethanol), as detailed in Table 2 and Figs. 5 to 11. The ANOVA

Table 2. IC_{50} values were calculated using a four-parameter nonlinear regression model. 95% confidence intervals were derived from the regression fit.

Sample	IC_{50} (μg/ml)	95% CI (Lower–Upper)	*p*-value vs. Control	Significance
Aerva lanata Q	84.10	80.50–87.80	< 0.001	***
Aerva lanata 6C	80.46	76.90–84.20	< 0.01	**
Aerva lanata 30C	114.10	108.20–120.40	< 0.05	*
Berberis vulgaris Q	188.00	178.40–197.60	< 0.01	**
Berberis vulgaris 6C	370.20	352.80–387.60	< 0.05	*
Berberis vulgaris 30C	332.90	315.40–350.40	> 0.05	ns
Hydrangea arborescens Q	90.99	86.40–95.60	< 0.05	*
Hydrangea arborescens 6C	126.60	120.20–133.00	> 0.05	ns
Hydrangea arborescens 30C	151.20	144.00–158.40	> 0.05	ns
Sarsaparilla Q	64.22	60.40–68.00	< 0.05	*
Sarsaparilla 6C	193.80	184.20–203.40	> 0.05	ns
Sarsaparilla 30C	250.00	238.40–261.60	> 0.05	ns
Ethanol (Control)	46.68	44.00–49.40	—	—

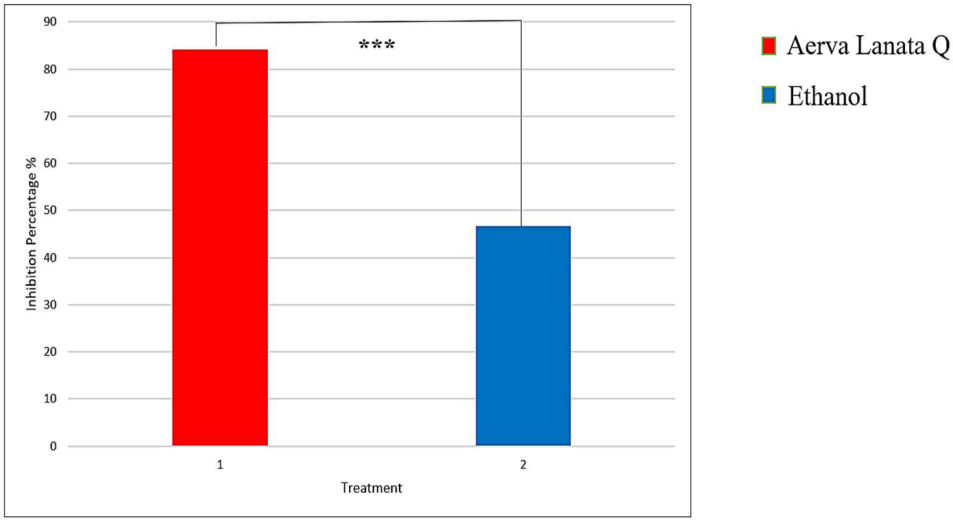


Fig. 5. Comparison of inhibition percentage between *Aerva lanata* Q and Ethanol treatments ($p < 0.001$). This highlights the statistical significance, indicated by the ***p-value < 0.001, suggesting a highly significant difference between the two treatments. Differences from control were tested by one-way ANOVA followed by Tukey's HSD test.

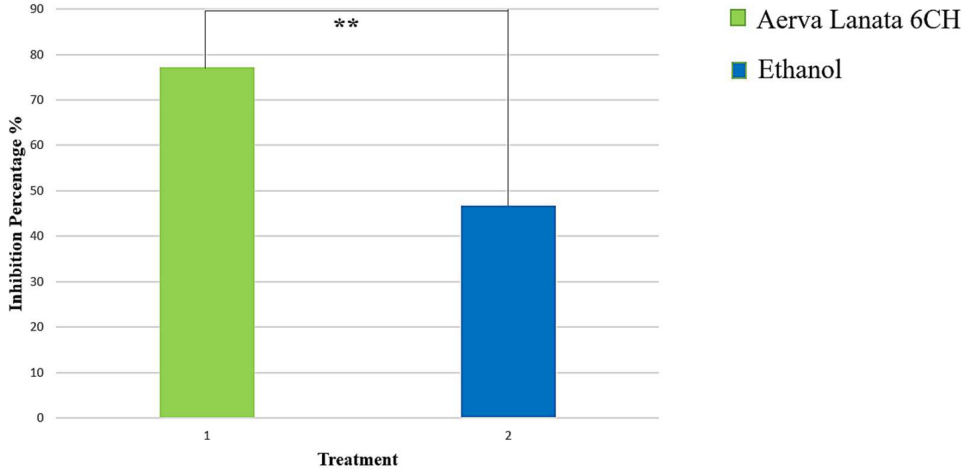


Fig. 6. Comparison of inhibition percentage between *Aerva lanata* 6C and Ethanol treatments ($p < 0.01$); This highlights the statistical significance, indicated by the **p-value less than 0.01, implying a highly significant difference between the two treatments. Differences from control were tested by one-way ANOVA followed by Tukey's HSD test.

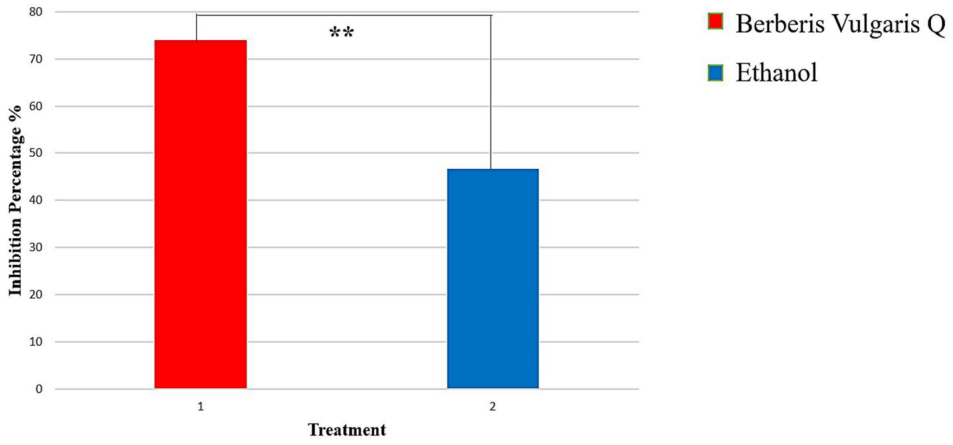


Fig. 7. Comparison of inhibition percentage between *Berberis vulgaris* Q and Ethanol treatments ($p < 0.01$); This highlights the statistical significance, indicated by the **p-value less than 0.01, implying a highly significant difference between the two treatments. Differences from control were tested by one-way ANOVA followed by Tukey's HSD test.

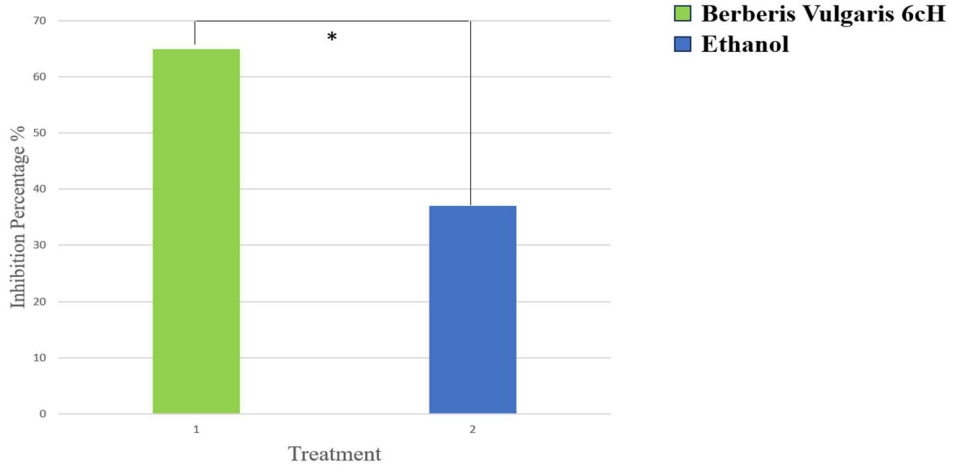


Fig. 8. Comparison of inhibition percentage between *Berberis vulgaris* 6C and Ethanol treatments ($p < 0.05$). This highlights the statistical significance, indicated by the *p-value < 0.05, suggesting a highly significant difference between the two treatments. Differences from control were tested by one-way ANOVA followed by Tukey's HSD test.

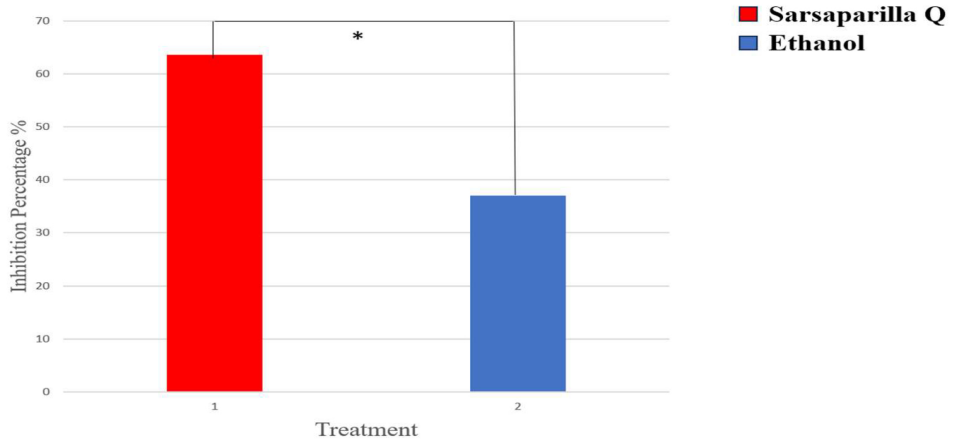


Fig. 9. Comparison of inhibition percentage between *Sarsaparilla* Q and Ethanol treatments ($p < 0.05$). This highlights the statistical significance, indicated by the *p-value < 0.05, suggesting a highly significant difference between the two treatments. Differences from control were tested by one-way ANOVA followed by Tukey's HSD test.

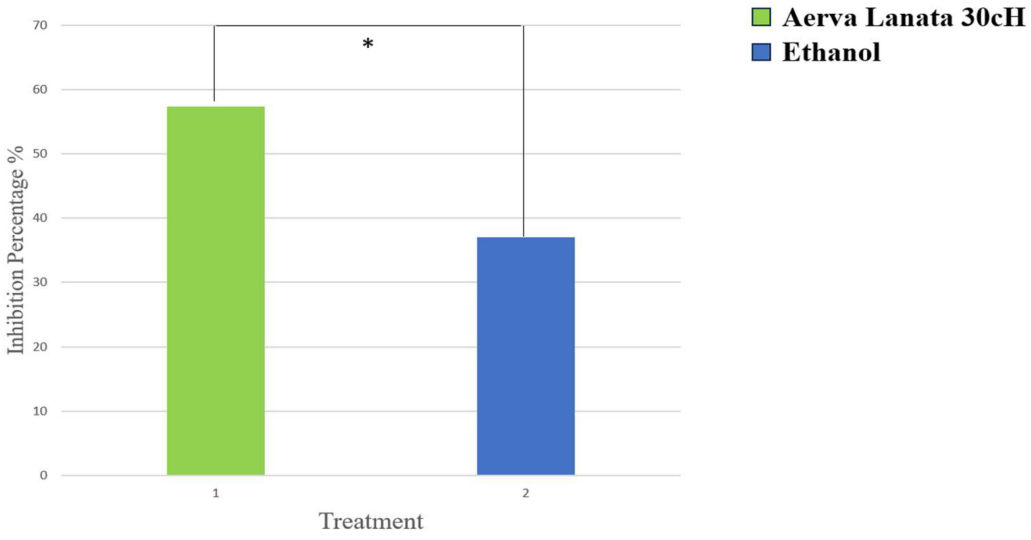


Fig. 10. Comparison of inhibition percentage between *Aerva Lanata* 30C and Ethanol treatments ($p < 0.05$). This highlights the statistical significance, indicated by the *p-value < 0.05, suggesting a highly significant difference between the two treatments. Differences from control were tested by one-way ANOVA followed by Tukey's HSD test.

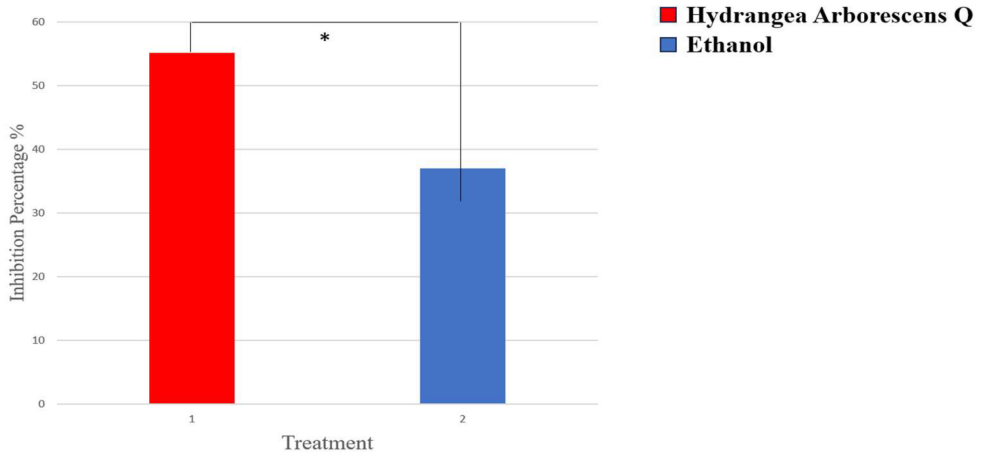


Fig. 11. Comparison of inhibition percentage between *Hydrangea arborescens* Q and Ethanol treatments ($p < 0.05$); This highlights the statistical significance, indicated by the *p-value less than 0.05, implying a highly significant difference between the two treatments. Differences from control were tested by one-way ANOVA followed by Tukey's HSD test.

and subsequent Tukey's HSD analysis showed that *Aerva lanata* Q, *Aerva lanata* 6C, and *Berberis vulgaris* Q had significantly higher inhibition of stone formation than the control. These results suggest that these remedies have potential anti-urolithiatic effects, with *Aerva lanata* Q being the most effective among the treatments tested. In Light Microscopic Studies slides treated with *Aerva lanata* (Q, 6C, 30C), *Berberis vulgaris* (Q, 6C, 30C), *Hydrangea arborescens* (Q, 6C, 30C), and *Sarsaparilla* (Q, 6C, 30C) at different concentrations is displayed markedly fewer crystals and reduced aggregation while comparing with Ethanol (90%) Tables 3 and 4. Notably, *Aerva lanata* Q, *Aerva lanata* 6C, and *Berberis vulgaris* Q showed greater aggregation than the control group (Ethanol), suggesting a potential anti-urolithiatic effect. Among the tested remedies, *Aerva lanata* Q emerged as the most effective in mitigating stone formation, highlighting its therapeutic promise in kidney stone prevention.

Discussion

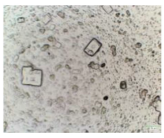
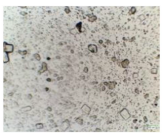
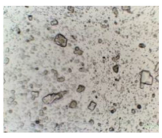
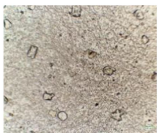
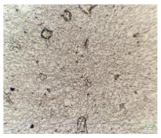
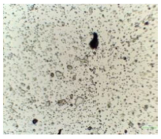
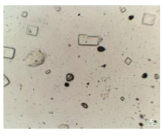
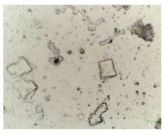
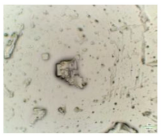
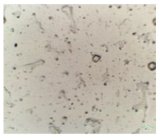
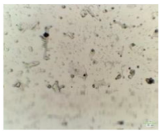
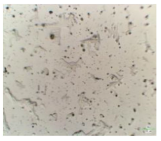
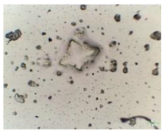
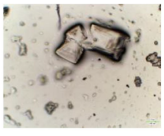
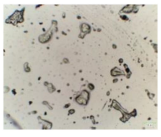
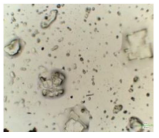
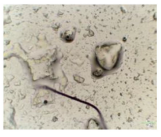
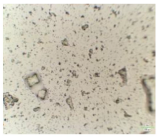
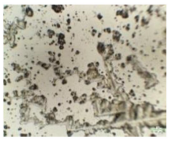
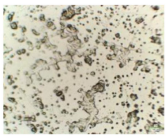
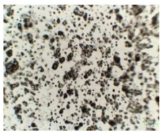
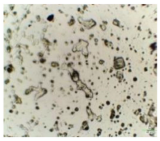
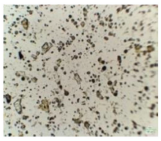
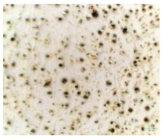
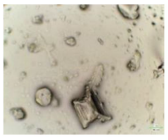
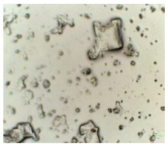
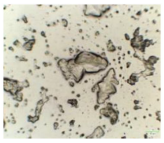
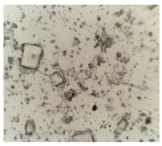
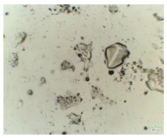
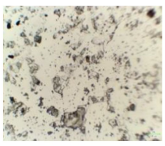
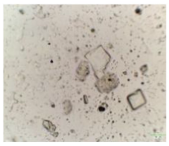
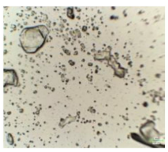
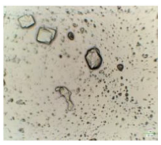
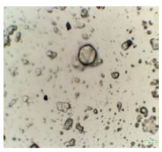
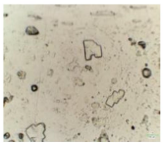
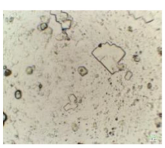
Calcium oxalate (CaOx) is the predominant type of kidney stone, and its crystallisation process plays a central role in stone formation. Inhibiting CaOx aggregation is therefore a key preventive strategy. In this study, *Aerva lanata* Q demonstrated

Table 3. Light microscopic observations (10× magnification) of CaOx crystal morphology following treatment with plant extracts at different concentrations.

Sample	IC_{50} (μg/ml)	95% CI (Lower–Upper)	p-value vs. Control	Significance
Aerva lanata Q	84.10	80.50–87.80	<0.001	***
Aerva lanata 6C	80.46	76.90–84.20	<0.01	**
Aerva lanata 30C	114.10	108.20–120.40	<0.05	*
Berberis vulgaris Q	188.00	178.40–197.60	<0.01	**
Berberis vulgaris 6C	370.20	352.80–387.60	<0.05	*
Berberis vulgaris 30C	332.90	315.40–350.40	>0.05	ns
Hydrangea arborescens Q	90.99	86.40–95.60	<0.05	*
Hydrangea arborescens 6C	126.60	120.20–133.00	>0.05	ns
Hydrangea arborescens 30C	151.20	144.00–158.40	>0.05	ns
Sarsaparilla Q	64.22	60.40–68.00	<0.05	*
Sarsaparilla 6C	193.80	184.20–203.40	>0.05	ns
Sarsaparilla 30C	250.00	238.40–261.60	>0.05	ns
Ethanol (Control)	46.68	44.00–49.40	—	—

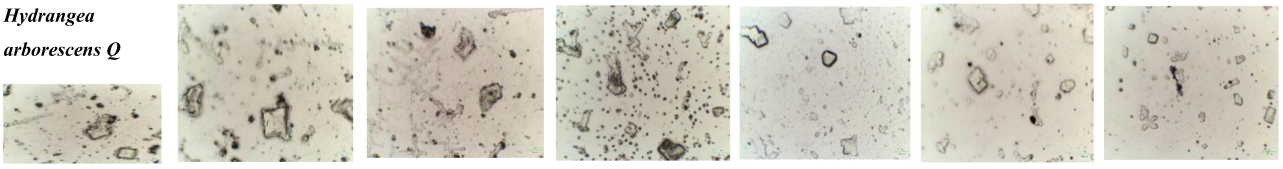
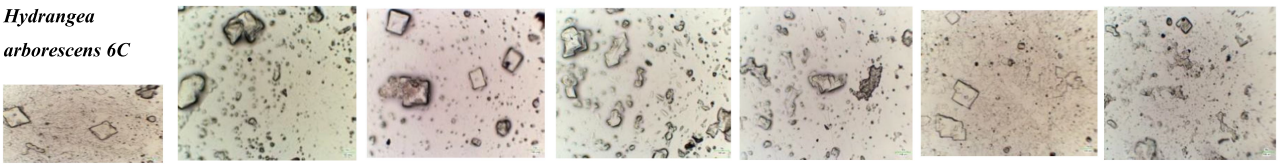
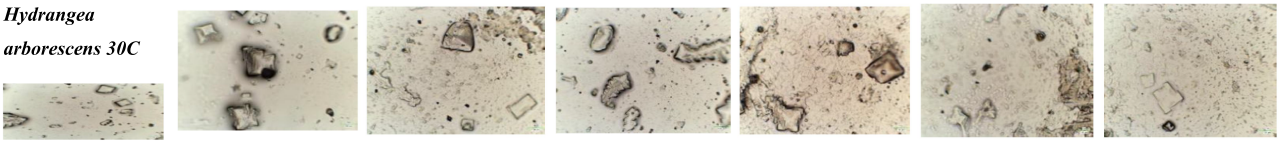
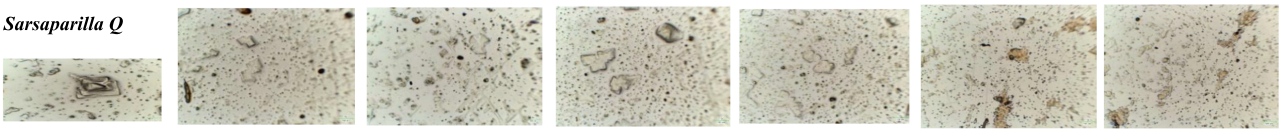
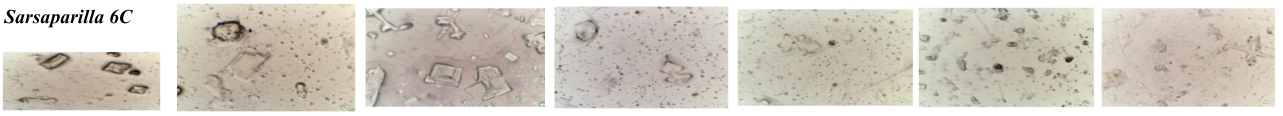
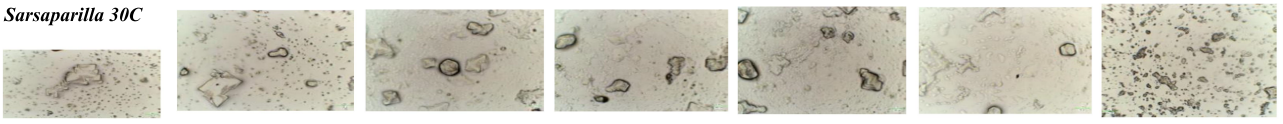
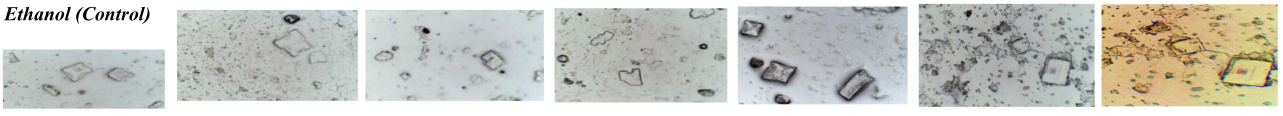
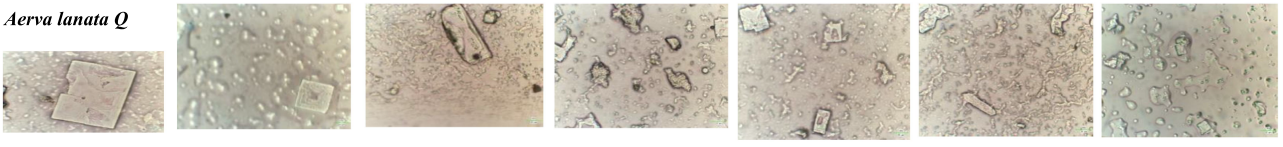
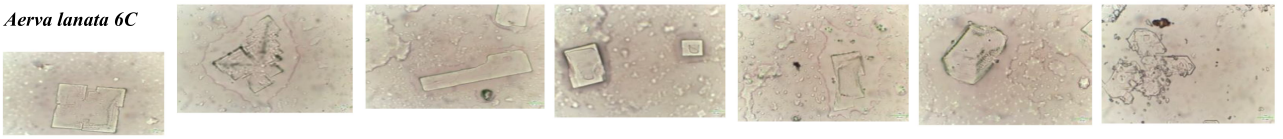
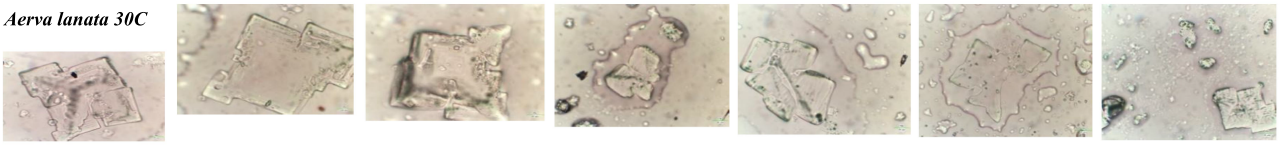
*Statistical comparisons with control (90% ethanol) were performed using one-way ANOVA followed by Tukey's HSD test. Significance: ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; ns = not significant.

Table 4. Light microscopic observations (40× magnification) of CaOx crystal morphology following treatment with plant extracts at different concentrations.

Medicine / Potency	10 µg/ml	50 µg/ml	100 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml
Aerva lanata Q						
Aerva lanata 6C						
Aerva lanata 30C						
Berberis vulgaris Q						
Berberis vulgaris 6C						
Berberis vulgaris 30C						

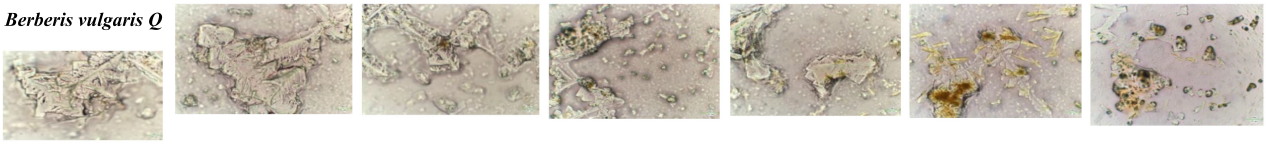
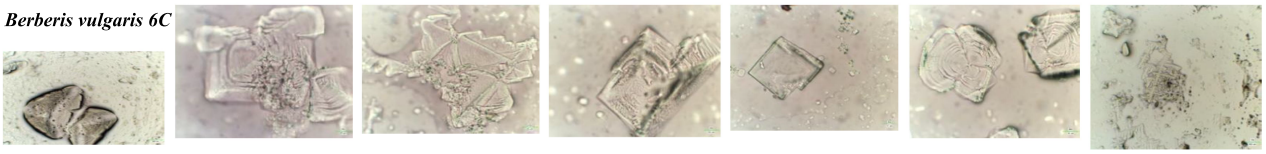
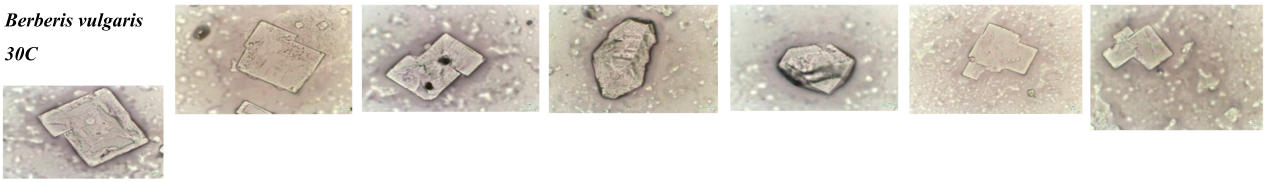
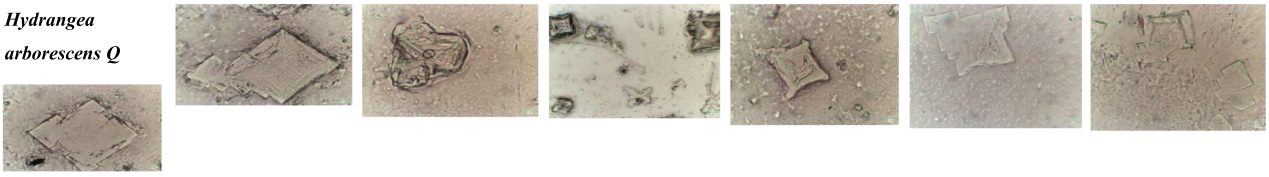
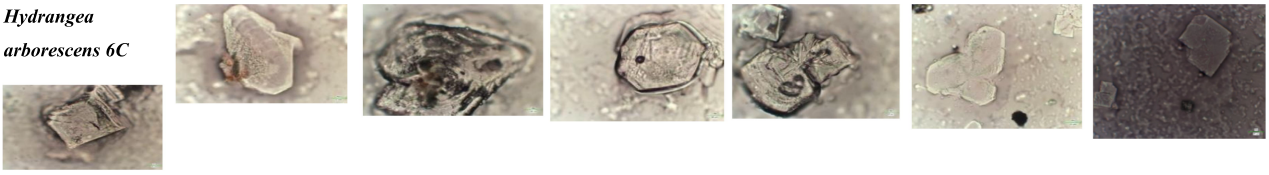
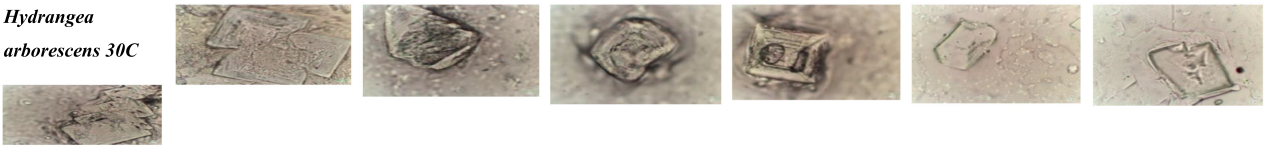
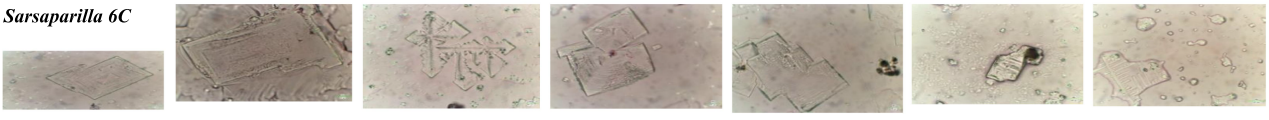
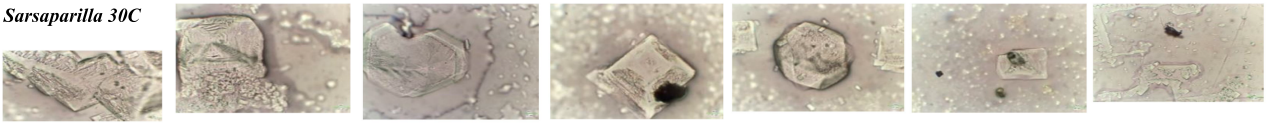
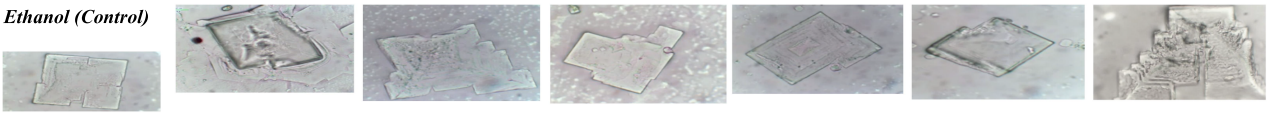
(Continued)

Table 4. Continued.

Medicine / Potency	10 µg/ml	50 µg/ml	100 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml
Hydrangea arborescens Q						
Hydrangea arborescens 6C						
Hydrangea arborescens 30C						
Sarsaparilla Q						
Sarsaparilla 6C						
Sarsaparilla 30C						
Ethanol (Control)						
Aerva lanata Q						
Aerva lanata 6C						
Aerva lanata 30C						

(Continued)

Table 4. Continued.

Medicine / Potency	10 µg/ml	50 µg/ml	100 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml
Berberis vulgaris Q						
Berberis vulgaris 6C						
Berberis vulgaris 30C						
Hydrangea arborescens Q						
Hydrangea arborescens 6C						
Hydrangea arborescens 30C						
Sarsaparilla Q						
Sarsaparilla 6C						
Sarsaparilla 30C						
Ethanol (Control)						

the strongest inhibitory effect, showing 86.48% inhibition at the highest concentration tested. This finding is consistent with earlier reports highlighting the flavonoid and alkaloid content of *Aerva lanata*, which interfere with crystal nucleation and aggregation.[5,6]

Similarly, *Berberis vulgaris* Q and 6C exhibited significant inhibition of CaOx aggregation, which may be attributed to the antioxidant and diuretic effects of berberine, known to reduce urinary supersaturation of calcium oxalate.[7] *Sarsaparilla* and *Hydrangea arborescens* showed moderate inhibition, supporting their traditional use but suggesting comparatively weaker effects under the in vitro conditions tested.

These results indicate that low potencies (Q and 6C) generally exhibited stronger activity than higher potencies (30C). This may reflect the presence of measurable phytoconstituents in Q and 6C preparations that directly interact with CaOx crystal formation, while higher dilutions may rely more on dynamic or indirect mechanisms. Comparable findings were reported by Patel *et al*. for *Sarsaparilla*, in which lower potencies showed more robust inhibitory effects on CaOx crystallisation.

Overall, our study supports the hypothesis that selected homoeopathic remedies, particularly *Aerva lanata* and *Berberis vulgaris*, have potential antiurolithiatic effects. However, the preliminary nature of this in vitro work must be emphasised. Future studies should incorporate advanced biochemical and spectroscopic assays (e.g., FTIR, Raman spectroscopy, oxalate quantification, and DLS) to confirm these mechanisms and strengthen translational relevance.

Conclusion

This study provides preliminary evidence that homoeopathic preparations, particularly *Aerva lanata* and *Berberis vulgaris*, exhibit inhibitory effects on calcium oxalate crystal aggregation in vitro. The highest inhibition was observed with *Aerva lanata* Q (86.48%) and *Berberis vulgaris* Q (73.95%). While these findings are encouraging, they cannot, by themselves, confirm anti-urolithiatic activity. The results should therefore be interpreted cautiously as an initial exploratory step. Future research must incorporate more precise biochemical assays (e.g., oxalate consumption and calcium quantification), spectroscopic methods (FTIR or Raman spectroscopy), and dynamic light scattering (DLS) to validate these effects, elucidate underlying mechanisms, and establish their clinical significance. Studies exploring higher potencies and other commonly used homoeopathic medicines will further strengthen the evidence base.

Acknowledgement

The authors are grateful to Trichy Research Institute of Biotechnology Pvt. Ltd., Trichy – 620018, Tamil Nadu for the technical support and facilities provided. We also thank Dr. K. Rajamanickam, Dr. A. Vettrivel and Dr. SP. Mary Adharshna for the support rendered.

Funding

Self funding.

Conflict of interest

None.

Author contribution

G. Loganathan and T. Satesh: Concepts, Design, Definition of intellectual content, Literature search, Clinical studies, Experimental studies, Data acquisition, Data analysis, Statistical analysis, Manuscript preparation, Manuscript editing, Manuscript review, and Guarantor.

References

1. Khan SR, Pearle MS, Robertson WG, *et al.* Kidney stones. *Nat Rev Dis Primers*. 2016;2(1):16008.
2. Romero V, Akpinar H, Assimos DG. Kidney stones: a global picture of prevalence, incidence, and associated risk factors. *Rev Urol*. 2010;12(2–3):e86–96.
3. Shirfule AL, Racharla V, Qadri SSYH, *et al.* Exploring antiurolithic effects of Gokshuradi Polyherbal Ayurvedic Formulation in ethylene-glycol-induced urolithic Rats. *Evid Based Complement Alternat Med*. 2013;2013:1–9.
4. Ghale-Salimi MY, Eidi M, Ghaemi N, *et al.* Inhibitory effects of taraxasterol and aqueous extract of *Taraxacum officinale* on calcium oxalate crystallisation: in vitro study. *Ren Fail.* 2018;40(1):298.
5. Kant R, Singh TG, Singh S. Mechanistic approach to herbal formulations used for urolithiasis treatment. *Obes Med.* 2020;19:100266.
6. Selvam R, Kalaiselvi P, Govindaraj A, *et al.* Effect of *A. Lanata* leaf extract and *vediuppu chunnam* on the urinary risk factors of calcium oxalate urolithiasis during experimental hyperoxaluria. *Pharmacol Res.* 2001;43(1):89–93.
7. Bashir S, Gilani AH, Siddiqui AA, *et al. Berberis vulgaris* root bark extract prevents hyperoxaluria induced urolithiasis in rats. *Phytother Res.* 2010;24(8):1250–5.
8. Nayak C, Sahoo A, Chaturbhuja N, *et al.* A case report of ureteric calculus treated with homoeopathic medicine, *Hydrangea arborescens 30*. *J Ayub Med Coll Abbottabad*. 2019;5:627–33.
9. Boericke DW, Boericke W. Pocket Manual of Homoeopathic Materia Medica & Repertory: Comprising the Characteristic

and Guiding Symptoms of All Remedies (clinical and Pathogenetic) Including Indian Drugs. B. Jain Publishers; 2002. 1208 p.
10. Telang R. An in vitro study of *Hydrangea arborescens*, homoeopathic preparation as an inhibitor of Calcium oxalate crystallisation. *Indian J Res Homoeopathy*. 2021;15(1): 24–30.
11. Taylor L. The Healing Power of Rainforest Herbs: A Guide to Understanding and Using Herbal Medicinals. Square One Publishers; 2005. 532 p.
12. R. Patel M, A. Khan T, Namdeo A, *et al*. In Vitro Study of Homoeopathic Medicine *Sarsaparilla* Q, 6c, 12c, 30c, 200c, 1m as an Inhibitor of Calcium Oxalate and Calcium phosphate Crystallisation. *Int. J. Sci. Res.* 2021;73–6.
13. Siddiqui V, Singh H, Gupta J, *et al.* A multicentre observational study to ascertain the role of homoeopathic therapy in Urolithiasis. *Indian J Res Homoeopathy*. 2026;20(1):30–44.
14. Zarin MA, Tan JS, Murugan P, *et al*. Investigation of potential anti-urolithiatic activity from different types of Musa pseudostem extracts in inhibition of calcium oxalate crystallisation. *BMC Complement Med Ther*. 2020;20(1):317.
15. Saha S, Verma RJ. Inhibition of calcium oxalate crystallisation in vitro by an extract of Bergenia ciliate. *Arab J Urol*. 2013;11(2):187–92.

Évaluation de l'activité anti-urolithiasique de différentes formulations de médicaments homéopathiques par le test de cristallisation de l'oxalate de calcium

Contexte : Les médicaments homéopathiques tels qu'*Aerva lanata, Hydrangea arborescens, Berberis vulgaris* et la *salsepareille* sont couramment utilisés pour traiter les calculs rénaux. Des recherches scientifiques sont nécessaires pour comprendre leurs effets sur la cristallisation de l'oxalate de calcium, un facteur clé de la lithiase urinaire. **Objectif :** L'étude a évalué les effets in vitro d'*Aerva lanata*, d'*Hydrangea arborescens*, de *Berberis vulgaris* et de la *salsepareille* aux dilutions Q, 6C et 30C sur la cristallisation de l'oxalate de calcium. Elle visait à identifier la dilution ayant le plus fort effet inhibiteur par rapport à l'éthanol (90 %). **Méthodes :** Des cristaux d'oxalate de calcium (CaOx) ont été synthétisés par mélange de chlorure de calcium et d'oxalate de sodium, puis refroidis à 37 °C pour obtenir une concentration de 0,8 mg/mL dans un tampon Tris (pH 6,5). Des remèdes homéopathiques (*Aerva lanata, Berberis vulgaris, Hydrangea arborescens* et *Salsaparilla* aux dilutions Q, 6C et 30C) et de l'éthanol (90 %) ont été testés à des concentrations de 1 000 à 10 µg/mL. Après 24 heures à 37 °C, la turbidité a été mesurée et la morphologie des cristaux a été examinée au microscope optique inversé. **Résultats :** *Aerva lanata* Q, *Aerva lanata* 6C et *Berberis vulgaris* Q ont inhibé significativement la formation de calculs par rapport au groupe témoin (éthanol), *Aerva lanata* Q présentant l'effet le plus marqué. La microscopie optique a révélé diverses morphologies cristallines, soulignant le rôle des cristaux d'oxalate de calcium dans la formation des calculs rénaux. **Conclusion :** *Aerva lanata* et *Berberis vulgaris* présentent un potentiel d'inhibition de la formation de cristaux d'oxalate de calcium, suggérant un rôle dans la prévention de la lithiase urinaire.

Screening der antiurolithiatischen Aktivität verschiedener homöopathischer Arzneimittelformulierungen mittels Calciumoxalat-Kristallisationstest

Hintergrund: Homöopathische Arzneimittel wie *Aerva lanata, Hydrangea arborescens, Berberis vulgaris* und *Sarsaparilla* werden häufig zur Behandlung von Nierensteinen eingesetzt. Wissenschaftliche Forschung ist erforderlich, um ihre Wirkung auf die Calciumoxalat-Kristallisation, einen Schlüsselfaktor bei Urolithiasis, zu verstehen. **Ziel:** Die Studie untersuchte die In-vitro-Wirkung von *Aerva lanata, Hydrangea arborescens, Berberis vulgaris* und *Sarsaparilla* in den Potenzen Q, 6C und 30C auf die Calciumoxalat-Kristallisation. Ziel war es, die Potenz mit der stärksten Hemmwirkung im Vergleich zu Ethanol (90 %) zu identifizieren. **Methoden:** Calciumoxalat-Kristalle (CaOx) wurden durch Mischen von Calciumchlorid und Natriumoxalat synthetisiert und anschließend auf 37 °C abgekühlt, um eine Konzentration von 0,8 mg/ml in Tris-Puffer (pH 6,5) zu erreichen. Homöopathische Mittel (*Aerva lanata, Berberis vulgaris, Hydrangea arborescens* und *Sarsaparilla* in den Potenzen Q, 6C und 30C) sowie Ethanol (90 %) wurden in Konzentrationen von 1000 bis 10 µg/ml getestet. Nach 24 Stunden bei 37 °C wurde die Trübung gemessen und die Kristallmorphologie unter einem inversen Lichtmikroskop untersucht. **Ergebnisse:** *Aerva lanata* Q, *Aerva lanata* 6C und *Berberis vulgaris* Q hemmten die Steinbildung im Vergleich zur Ethanol-Kontrollgruppe signifikant, wobei *Aerva lanata* Q die stärkste Wirkung zeigte. Die Lichtmikroskopie zeigte vielfältige Kristallmorphologien und unterstrich damit die Rolle von Calciumoxalatkristallen bei der Nierensteinbildung. **Schlussfolgerung:** *Aerva lanata* und *Berberis vulgaris* zeigen Potenzial bei der Hemmung der Calciumoxalatkristallbildung und könnten somit zur Prävention von Urolithiasis beitragen.

कैल्शियम ऑक्सालेट क्रिस्टलीकरण परीक्षण का उपयोग करके होम्योपैथिक दवाओं के विभिन्न फॉर्मूलेशन की एंटीयूरोलिथिएटिक गतिविधि की स्क्रीनिंग

पृष्ठभूमि: *एर्वा लानाटा, हाइड्रेंजिया आर्बोरेसेंस, बर्बेरिस वल्गारिस* और *सरसापारिला* जैसी होम्योपैथिक दवाओं का उपयोग आमतौर पर गुर्दे की पथरी के इलाज के लिए किया जाता है। यूरोलिथियासिस में एक प्रमुख कारक, कैल्शियम ऑक्सालेट क्रिस्टलीकरण पर उनके प्रभावों को समझने के लिए वैज्ञानिक अनुसंधान की आवश्यकता है। **उद्देश्य:** इस अध्ययन में *एर्वा लानाटा, हाइड्रेंजिया आर्बोरेसेंस, बर्बेरिस वल्गारिस* और *सरसापारिला* के Q, 6C और 30C पोटेंसी में कैल्शियम ऑक्सालेट क्रिस्टलीकरण पर इन विट्रो प्रभावों का मूल्यांकन किया गया। इसका उद्देश्य यह पहचानना था कि किस पोटेंसी का एथेनॉल (90%) की तुलना में सबसे मजबूत निरोधात्मक प्रभाव है। **विधि:** कैल्शियम क्लोराइड और सोडियम ऑक्सालेट को मिलाकर कैल्शियम ऑक्सालेट (CaOx) क्रिस्टल संश्लेषित किए गए, फिर उन्हें 37°C तक ठंडा करके ट्रिस बफर (pH 6.5) में 0.8 मिलीग्राम/मिलीलीटर की सांद्रता प्राप्त की गई। होम्योपैथिक औषधियाँ *(एर्वा*

लानाटा, बर्बेरिस वल्गारिस, हाइड्रेंजिया आर्बोरिसेंस और *सरसापारिला,* क्रमशः Q, 6C और 30C पोटेंसी में) और इथेनॉल (90%) का 1000 से 10 माइक्रोग्राम/मिलीलीटर की सांद्रता पर परिक्षण किया गया। 37°C पर 24 घंटे बाद, धुंधलेपन को मापा गया और क्रिस्टल की आकृति का अध्ययन इनवर्टेड लाइट माइक्रोस्कोप के अंतर्गत किया गया। **परिणाम:** *एर्वा लानाटा* Q, *एर्वा लानाटा* 6C और *बर्बेरिस वल्गारिस* Q ने इथेनॉल नियंत्रण समूह की तुलना में पथरी बनने को काफी हद तक रोका, जिसमें *एर्वा लानाटा* Q का प्रभाव सबसे अधिक स्पष्ट था। लाईट माइक्रोस्कोप से विभिन्न क्रिस्टल संरचनाओं का पता चला, जो गुर्दे की पथरी के निर्माण में कैल्शियम ऑक्सालेट क्रिस्टल की भूमिका को रेखांकित करता है। **निष्कर्ष:** *एर्वा लानाटा* और *बर्बेरिस वल्गारिस* कैल्शियम ऑक्सालेट क्रिस्टल निर्माण को रोकने में संभावित रूप से सक्षम हैं, जो मूत्र पथरी की रोकथाम में इनकी भूमिका का संकेत देता है।

Detección de la actividad antiurolitiásica de diferentes formulaciones de medicamentos homeopáticos mediante el ensayo de cristalización de oxalato de calcio

Antecedentes: Los medicamentos homeopáticos como *Aerva lanata, Hydrangea arborescens, Berberis vulgaris* y *Sarsaparilla* se utilizan comúnmente para tratar los cálculos renales. Se necesita investigación científica para comprender sus efectos sobre la cristalización del oxalato de calcio, un factor clave en la urolitiasis. **Objetivo:** El estudio evaluó los efectos in vitro de *Aerva lanata, Hydrangea arborescens, Berberis vulgaris* y *Sarsaparilla* en potencias Q, 6C y 30C sobre la cristalización del oxalato de calcio. Su objetivo fue identificar qué potencia tenía el mayor efecto inhibidor en comparación con el etanol (90 %). **Métodos:** Se sintetizaron cristales de oxalato de calcio (CaOx) mezclando cloruro de calcio y oxalato de sodio, y luego se enfriaron a 37 °C para lograr una concentración de 0,8 mg/mL en tampón Tris (pH 6,5). Se probaron remedios homeopáticos (*Aerva lanata, Berberis vulgaris, Hydrangea arborescens* y *Sarsaparilla* en potencias Q, 6C y 30C) y etanol (90 %) en concentraciones de 1000 a 10 µg/ml. Después de 24 horas a 37 °C, se midió la turbidez y se examinó la morfología de los cristales bajo un microscopio óptico invertido. **Resultados:** *Aerva lanata* Q, *Aerva lanata* 6C y *Berberis vulgaris* Q inhibieron significativamente la formación de cálculos en comparación con el grupo control de etanol, siendo *Aerva lanata* Q la que demostró el efecto más pronunciado. La microscopía óptica reveló diversas morfologías cristalinas, lo que subraya el papel de los cristales de oxalato de calcio en la formación de cálculos renales. Conclusión: *Aerva lanata* y *Berberis vulgaris* muestran potencial para inhibir la formación de cristales de oxalato de calcio, lo que sugiere su papel en la prevención de la urolitiasis.

利用草酸鈣結晶試驗篩選不同順勢療法藥物製劑的抗泌尿道結石活性

背景：順勢療法藥物，如毛葉菽草（*Aerva lanata*）、繡球花（*Hydrangea arborescens*）、小蘗（*Berberis vulgaris*）和菽草（*Sarsaparilla*），常用於治療腎結石。需要進行科學研究以了解它們對草酸鈣結晶的影響，而草酸鈣結晶是尿路結石的關鍵因素。**目的**：本研究評估了毛葉菽草、繡球花、小蘗和菽草在Q、6C和30C效價下對草酸鈣結晶的體外作用。旨在確定與乙醇（90%）相比，哪種效價具有最強的抑製作用。**方法**：將氯化鈣和草酸鈉混合，合成草酸鈣（CaOx）晶體，然後冷卻至37℃，使其在Tris緩衝液（pH 6.5）中達到0.8 mg/mL的濃度。測試了濃度為1000至10 µg/mL的順勢療法藥物（*Aerva lanata*、*Berberis vulgaris*、*Hydrangea arborescens*和*Sarsaparilla*，效價分別為Q、6C和30C）和乙醇（90%）。在37℃下孵育24小時後，測量濁度，並在倒置顯微鏡下觀察晶體形態。結果：與乙醇對照組相比，*Aerva lanata* Q、*Aerva lanata* 6C和*Berberis vulgaris* Q均能顯著抑制結石形成，其中*Aerva lanata* Q的效果最為顯著。光學顯微鏡觀察顯示晶體形態多樣，突顯了草酸鈣結晶在腎結石形成中的作用。**結論**：毛葉菽草（*Aerva lanata*）和歐洲小蘗（*Berberis vulgaris*）具有抑制草酸鈣結晶形成的潛力，提示其在預防泌尿道結石方面可能發揮作用。